SUPPLEMENTARY MATERIAL

Plasma Aβ42/40 ratios as biomarkers for Aβ cerebral deposition in cognitively normal individuals.

I. IMAGING METHODS

Aβ-amyloid imaging was performed with two different radiotracers: ^{11}C-Pittsburgh Compound-B (PiB) and ^{18}F-flutemetamol (FLUTE). The PET methodology for each tracer has been previously described [1;2]. For semi-quantitative analysis, a volume of interest template was applied to the summed and spatially normalized PET images in order to obtain a standardized uptake value (SUV). The images were then scaled to the SUV of each tracer recommended reference region to generate a tissue ratio termed SUV ratio (SUVR). A cortical measure of Aβ burden was computed using the mean SUVR in the frontal, superior parietal, lateral temporal, lateral occipital, and anterior and posterior cingulate regions. For PiB, the SUVs were normalized to the cerebellar cortex and the pons was used as the reference region for FLUTE [3]. In order to use the results of both PET tracers as a single continuous variable, FLUTE results were transformed into PiB-like SUVR termed BeCKeT [4]. The SUVR/BeCKeT was then dichotomized into high (Aβ+) or low (Aβ-) Aβ burden using a ≥1.5 SUVR/BeCKeT cut-off [4].

II. STATISTICAL METHODS

Details of the cross-validation experiment for ROC analysis

A cross-validation experiment was designed in order to assess the performance of predicting dichotomic SUVR/BeCKeT. The classifier was based on a linear regression model including the plasma ratio log(TP42/40), and the age and the APOE4 status as covariates. In order to maximize the sample size, all plasma-PET data pairs from visits m18 to m54 were selected for this experiment since a large enough amount of data is required for both training and testing sets, in order to obtain a smooth and reliable estimation of the ROC curve.

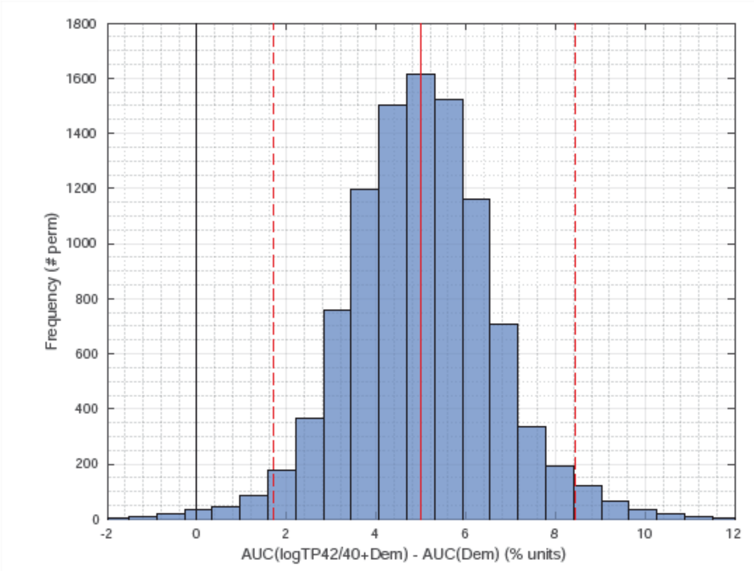
When using cross-validation for assessing classification performance, caution must be taken to avoid bias. In this experiment, it was ensured that for each cross-validation round all measurements from each subject were either in the training or in the testing set, i.e., the random selection for either training or testing sets was performed at subject level. Additionally, in order to get a more reliable estimation of the classification metrics, the sample prevalence, i.e. an equal proportion of PET amyloid β positive subjects in each random data sampling was purposefully maintained similar to the sample prevalence in the complete sample of the study (≈40%).

In each cross-validation round, the testing set consisted of 117 measurement-pairs from 39 subjects with valid measurements at all three visits. Sixteen out of these 39 subjects for testing were randomly selected among the set of amyloid-PET positive subjects. The remaining 23 subjects were randomly selected from the Aβ-PET negative subjects. In this way, the sample prevalence in the testing set was 41% for each CV round.

III. CLASSIFICATION PERFORMANCE DIFFERENCES BETWEEN THE MODEL WITH AND WITHOUT TP42/40

AUC

In the cross-validation experiment, each of the random splitting of the data was used to assess both models, with and without the plasma marker TP42/40. Accordingly, direct comparison of the classification performance metrics (such as AUC, sensitivity, specificity, PPV, etc.) from both models at each of the cross-validation rounds was carried out. **Supplementary Fig. 1** illustrates the histogram of the AUC difference between both models: AUC of the model including log(TP42/40) and the model only including Age+APOE4 as explanatory variables. The difference in AUC was found to be statistically significant, with an average difference of 5%, and a 95% confidence interval of (1.70-8.42). Only at 64 out of the 10,000 permutations, the AUC of the demographic model was equal or higher than the AUC of the model including the plasma marker TP42/40, so the inclusion of TP42/40 provides a significant improvement in AUC comparing with the model with only age and APOE4.

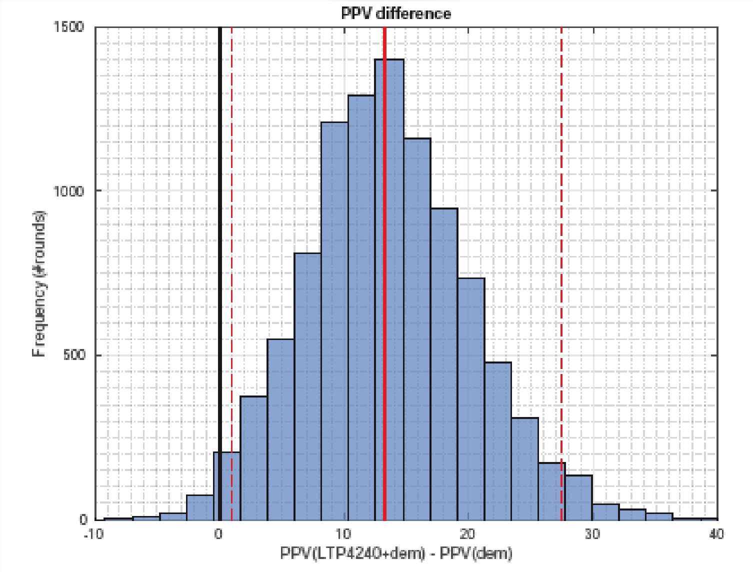


Supplementary Fig. 1. Histogram of the difference of the AUC of the model including log(TP42/40) and the AUC with only age+APOE4 variables in the cross-validation experiment. The red vertical lines represent the distribution percentiles: solid line for 50% (median value) and dashed lines for 2.5% and 97.5% percentile values.

In addition to this non-parametric estimation of the performance difference, DeLong test was used to assess the AUC difference between the models with and without the plasma marker, providing a *P* value of .0017. These findings show a strong agreement between the result of DeLong test and the estimation with the cross-validation experiment, which confirms the significant improvement in AUC with the inclusion of TP42/40 in the model.

PPV

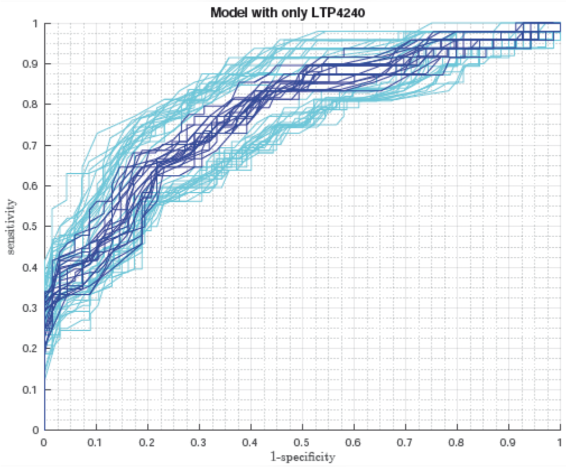
At each cross-validation round, the threshold value of the classifier was chosen in order to achieve a given value of sensitivity. The difference in the PPV using those threshold values are shown in **Supplementary Fig. 2**. The improvement in PPV was found to be statistically significant, showing an average effect-size of 13.3%, and a 95% confidence interval in the improvement of (1.02-27.43%). A positive PPV difference was observed in 9,798 rounds out of 10,000 rounds performed in the cross-validation experiment, providing a probability of 97.98%.

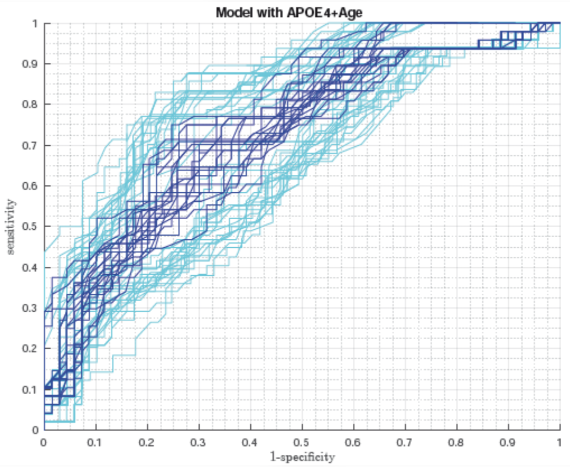


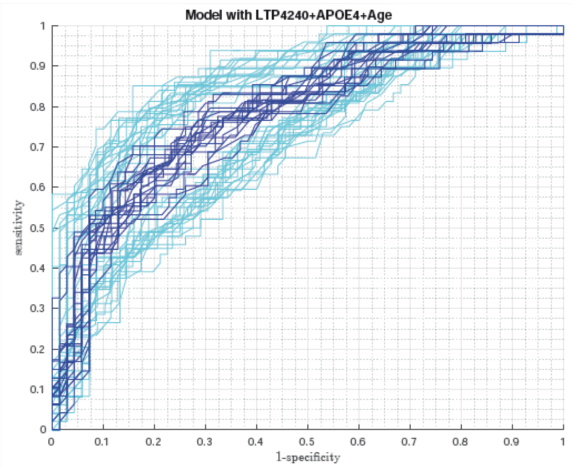
Supplementary Fig. 2. Comparison of the performance difference of PPV between the classifiers with/without the plasma marker log(TP42/40) as a function of the threshold value. The thick red line represents the median value of the performance increment, and the thin red lines the 2.5th and the 97.5th percentile values of the distribution.

IV. ROC CURVES COMPARISON

Illustrative ROC curves in the cross-validation experiment are shown in **Supplementary Fig. 3.** The average and percentile values of the AUC from the three classifiers are given in the table below.







Average and percentile values of the AUC of the three classifiers

Model	Average	5th prct	50th prct	95th prct
LTP4240	78.73	72.77	78.65	84.92
Age+APOE4	73.95	66.28	74.03	81.46
LTP4240+Age+APOE4	78.94	72.67	79.00	85.11

Supplementary Fig. 3. Distribution of ROC curves obtained in the cross-validation experiment for the three classifiers: (top-left) with only log(TP42/40) marker; (top-right) with demographic variables Age+APOE4; (bottom-left) with plasma + demographic variables. Representative ROC curves show the performance variability across cross-validation (CV) rounds. The variability is caused by the limited size of the datasets used for training and testing. A different ROC curve is obtained for each CV round. Curves whose AUC are between percentiles 49.9% and 50.1% are shown in blue color, while cyan is used for ROC curves in the percentile intervals (2.4%–2.6%) and (97.4%–97.6%).

Supplementary Table 1. Coefficient estimates, 95% confidence interval and significance of the linear regression models for each visit in the cross-sectional study.

	Visit m18			Visit m36			Visit m54		
	Estimate	CI 95%	*P* value	Estimate	CI 95%	*P* value	Estimate	CI 95%	*P* value
Intercept	-0.7	-2, 0.62	0.29	-0.18	-1.4, 1	0.77	-0.7	-1.9, 0.53	0.26
log(TP42/40)	-0.4	-0.76, -0.027	**0.036**	-0.1	-0.43, 0.22	0.52	-0.59	-0.94, -0.24	**0.0013**
Age	0.013	4.6e-05, 0.027	**0.049**	0.016	0.0014, 0.031	**0.033**	0.0076	-0.0055, 0.021	0.235
APOE4 carrier	0.31	0.11, 0.52	**0.0033**	0.36	0.14, 0.59	**0.00224**	0.28	0.086, 0.47	**0.005**
Gender Male	0.15	-0.039, 0.35	0.12	0.18	-0.027, 0.39	0.086	0.03	-0.15, 0.21	0.75

	Visit m18			Visit m36			Visit m54		
	Estimate	CI 95%	*P* value	Estimate	CI 95%	*P* value	Estimate	CI 95%	*P* value
Intercept	0.038	-1.2, 1.3	0.95	-0.45	-1.6, 0.66	0.42	0.11	-0.97, 1.2	0.84
log(BP42/40)	-0.086	-0.32, 0.14	0.46	-0.29	-0.51, -0.066	**0.012**	-0.3	-0.54, -0.065	**0.013**
Age	0.014	-0.001, 0.029	0.067	0.014	-0.00014, 0.028	0.052	0.0067	-0.0072, 0.021	0.34
APOE4 carrier	0.34	0.11, 0.56	**0.0042**	0.37	0.16, 0.58	**0.00095**	0.27	0.074, 0.47	**0.0078**
Gender Male	0.15	-0.055, 0.36	0.15	0.12	-0.088, 0.32	0.26	0.043	-0.15, 0.23	0.65

	Visit m18			Visit m36			Visit m54		
	Estimate	CI 95%	*P* value	Estimate	CI 95%	*P* value	Estimate	CI 95%	*P* value
Intercept	-0.65	-1.9, 0.65	0.32	-0.26	-1.7, 1.1	0.71	-0.51	-1.9, 0.91	0.48
log(FP42/40)	-0.35	-0.63, -0.067	**0.016**	-0.055	-0.36, 0.25	0.72	-0.32	-0.64, 0.0027	0.052
Age	0.014	-0.00041, 0.028	0.057	0.018	0.0039, 0.033	**0.014**	0.013	-5.8e-05, 0.027	**0.047**
APOE4 carrier	0.33	0.11, 0.54	**0.0033**	0.42	0.2, 0.63	**0.00029**	0.31	0.12, 0.51	**0.0021**
Gender Male	0.13	-0.071, 0.33	0.2	0.19	-0.014, 0.39	0.067	0.077	-0.11, 0.27	0.42

Regression models of the cross-sectional association of plasma Aβ ratios as log-transformed continuous variables and the SUVR/BeCKeT, adjusted for age, APOE genotype and gender. The estimate column refers to the corresponding coefficient of the particular plasma variable or the selected covariates in the model. CI 95%: 95% confidence interval.

Supplementary Table 2. Coefficients, 95% confidence interval and significance of the linear mixed-effects models in the longitudinal study.

		CI 95%		
	Estimate	Lower	Upper	*P* value
Intercept	-0.79	-1.8	0.17	0.1
Time	-0.098	-0.17	-0.028	0.0063
log(TP42/40)	**-0.34**	**-0.62**	**-0.064**	**0.016**
Age	0.016	0.0062	0.027	0.0017
APOE4 carrier	0.32	0.17	0.48	4.4e-05
Gender Male	0.076	-0.068	0.22	0.3
log(TP42/40):Time	**-0.034**	**-0.054**	**-0.015**	**0.0006**
Age:Time	0.00016	-0.00051	0.00083	0.64
APOE4 carrier:Time	0.018	0.0069	0.029	0.0016
Gender Male:Time	0.0012	-0.0095	0.012	0.82

		CI 95%		
	Estimate	Lower	Upper	*P* value
Intercept	-0.39	-1.3	0.53	0.4
Time	-0.045	-0.11	0.022	0.19
log(BP42/40)	**-0.13**	**-0.31**	**0.047**	**0.15**
Age	0.018	0.0073	0.029	0.0012
APOE4 carrier	0.34	0.18	0.51	4.6e-05
Gender Male	0.07	-0.083	0.22	0.37
log(BP42/40):Time	**-0.014**	**-0.028**	**-0.00094**	**0.036**
Age:Time	8.9e-05	-0.00066	0.00083	0.81
APOE4 carrier:Time	0.019	0.007	0.031	0.0021
Gender Male:Time	0.002	-0.0092	0.013	0.73

		CI 95%		
	Estimate	Lower	Upper	*P* value
Intercept	-0.47	-1.4	0.47	0.32
Time	-0.084	-0.16	-0.0091	0.028
log(FP42/40)	**-0.22**	**-0.43**	**0.0045**	**0.055**
Age	0.016	0.0051	0.027	0.0042
APOE4 carrier	0.33	0.17	0.5	5.9e-05
Gender Male	0.054	-0.097	0.21	0.48
log(FP42/40):Time	**-0.026**	**-0.042**	**-0.0098**	**0.0017**
Age:Time	0.00023	-0.00052	0.00097	0.55
APOE4 carrier:Time	0.018	0.0061	0.03	0.0033
Gender Male:Time	0.0012	-0.01	0.012	0.84

Estimate: coefficient of the adjusted LMM including baseline levels of TP42/40, BP42/40 or FP42/40 as explanatory variables in each case and the SUVR/BeCKeT as a continuous response variable during follow-up. In bold, the magnitude of the effect (estimate) of the different Aβ plasma ratios on the intercept (without interaction with time) and on the slope (ratio:Time) of the SUVR/BeCKeT score trajectory . CI 95%: lower and upper limits of the 95% confidence interval. The demographic covariates are APOE genotype, age and gender. As could be expected, the age and the APOE genotype had significant effects (estimate) on the baseline levels of SUVR/BeCKeT. Furthermore, the presence of one ApoE ε4 allele also had a significant effect on the trajectory of SUVR/BeCKeT (slope) over time (APOE4 carrier:Time).

Reference List

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